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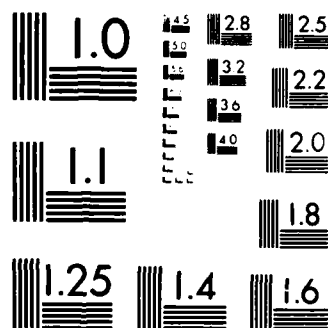
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FEASIBILITY OF HUMAN SKIN GRAFTS ON AN ISOLATED  
BUT ACCESSIBLE VASCULAR SUPPLY ON ATHYMIC RATS  
AS A SYSTEM TO STUDY PERCUTANEOUS  
PENETRATION AND CUTANEOUS INJURY

ANNUAL SUMMARY REPORT

GERALD G. KRUEGER, M.D. Principal Investigator  
SUE E. HUETHER, PhD. Co-investigator

NOVEMBER 1983

Supported By

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012

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University of Utah  
Salt Lake City, Utah 84132

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causing the rejection process. Grafts have remained viable in three animals for 12-16 weeks indicating the rejection problem may be resolved.

An evaluation of blood flow dynamics has been undertaken since blood flow is significantly related to rates of percutaneous absorption and skin responses to cutaneous injury. Volume blood flow measurement through the vessels supplying the flap is being established by use of C-type electromagnetic flow probes, which are applied to the outside of the vessel. Preliminary measures in Lewis rats indicate the flow rate is between 1.0 and 2.5 ml per minute. The evaluation of skin blood flow at control sites and on both sides of the flap is being assessed using laser Doppler velocimetry. LDV values at abdominal control sites are approximately 300-350 m.v. Blood flow to the host side of the graft is less than the control site and approximately 60% greater than the human skin graft. Blood flow to the flap is more than required for maintaining flap viability. Investigations manipulating blood flow through the flap are being initiated and include an evaluation of blood flow change with changes in body and flap temperature, and use of vasoactive agents.

Histologic techniques for assessment of cellular responses to cutaneous injury are in process. The techniques are related to quantitatively assessing epidermal DNA synthesis.

Continuing experimental objectives have been projected to include:

- a. Defining relationships between electromagnetic flow values, LDV flow velocity values, and systemic blood pressure.
- b. Describing the morphology of the model flap circulation.
- c. Determining the effects of repeated blood sampling on vessel structure and blood flow.
- d. Defining the rejection process of the human skin grafts by the nude rat.
- e. Evaluate relationships between percutaneous absorption, cutaneous injury, and different thickness and types (facial vs abdominal) of skin.
- f. Quantitate responses to cutaneous injury.



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## Summary

A model for grafting human skin to a neurovascular island flap is being developed on congenitally athymic nude rats. Success with the model will provide a human skin surface for the study of chemical warfare agents.

Applying microsurgery techniques, a neurovascular island flap using syngeneic skin has been successfully established in both the Lewis and nude rats. Early experimentation using human skin for the flap model in nude rats resulted in rejection. The immunologic mechanisms responsible for the rejection phenomenon are unknown. A series of experiments has been initiated to define factors causing the rejection process. Grafts have remained viable in three animals for 12-16 weeks indicating the rejection problem can be resolved.

An evaluation of blood flow dynamics has been undertaken since blood flow is significantly related to rates of percutaneous absorption and skin responses to cutaneous injury. Volume blood flow measurement through the vessels supplying the flap is being established by use of C-type electromagnetic flow probes which are applied to the outside of the vessel. Preliminary measures in Lewis rats indicate the flow rate is between 1.0 and 2.5 ml per minute. The evaluation of skin blood flow at control sites and on both sides of the flap is being assessed using laser Doppler velocimetry (LDV). LDV values at abdominal control sites is approximately 300-350 m.v. Blood flow to the host side of the graft is less than the control site and approximately 60% greater than the skin graft. Blood flow to the flap is more than that required for maintaining flap viability. Investigations manipulating blood flow through the flap are being initiated and include an evaluation of blood flow change with changes in body and flap temperature, and use of vasoactive agents.

Histologic techniques for assessment of cellular responses to cutaneous injury are in process. The techniques are related to quantitatively assessing epidermal DNA synthesis.

Continuing experimental objectives have been projected to include:

- a. Definition of the relationships between electromagnetic flow values, LDV flow velocity values, and systemic blood pressure.
- b. Descriptions of the morphology of the circulation of the model flap.
- c. Determination of the affects of repeated blood sampling on vessel structure and blood flow.
- d. Definition of the rejection process of the human skin grafts by the nude rat.
- e. Evaluation of the relationships between percutaneous absorption, cutaneous injury, and different thickness and types (facial vs abdominal) of skin.
- f. Quantitation of responses to cutaneous injury.

## Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978). For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.



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### Problem Statement

The problem of this study is to determine if human skin can be successfully grafted to a neurovascular island flap on congenitally athymic nude rats (rnu/rnu). The development of such a model will provide a system for the study of agents related to chemical warfare.

### Background Information

Background information is presented within each section of the report.

### Objectives and Accomplishments

Project objectives and the results of experimentation for the 01 year are presented in detail below.

I. Acquire personnel to complete the objectives of the project.

The following personnel are currently participating in the program:

#### A. Professional Personnel:

Gerald G. Krueger, M.D., Principal Investigator, Percent Effort: 28%, recently increased to 55%.

Sue E. Huether, PhD, Micro-Circulation Physiologist, 20% effort

Larry G. Leonard, M.D., Micro-Vascular Surgeon, 10% effort

Michael W. Piepkorn, M.D., PhD, Dermatopathologist, 5% effort

#### B. Non-Professional Personnel:

Current employees:

Zbigniew Wojciechowski, M.D., Micro-Vascular Surgeon, 100% effort

Judy Corlett, Animal Colony Handler/Research Technician, 100% effort

Steven Heath, Research Assistant, 100% effort

Bernard LaSalle, Senior Research Assistant, 20% effort

Others:

Doreen Hardie, Pre-Doctoral Student, Micro-Circulation

Scott Burton, Post-Doctoral Student in Percutaneous Pharmacology

Amos Gilhar, Research Dermatologist, to begin 1 January 1984

## II. Establish housing for the breeding and protection of the nude rat colony.

Space was acquired in the basement of building 419 in the Health Sciences area of the University of Utah campus. Remodeling of the animal housing room was completed by December 1, 1982. Remodeling included construction to make the area rodent and insect impermeable; the placement of a positive airflow system; and a floor and wall treatment finish which can be antiseptically maintained. Laminar airflow systems with carts and cages were delivered and operational by mid December.

## III. Establish a colony of nude rats.

A colony of nude rats (Rowett strain) was started with eight females and four males. Mating commenced about November 1, 1982, with the subsequent delivery of several litters. A number of parameters were found to be necessary to maintain a stable colony. These include: preventing changes in environmental temperature; appropriate nesting material in the cages; changing the composition of the water; and adding vitamin supplements to the diet. At the present time our breeding colony includes animals obtained from the Stanford Research Institute (Lewis strain), National Cancer Institute and National Institutes of Health (Outbred strain), Sprague-Dawley Corporation (Rowett strain). We have continued to successfully breed these animals and have an ample population for developing the human-rat skin flap model. A summary of the rat colony population as of 10 October 83 is presented in Table A.

## IV. Develop the surgical technology for grafting human skin to a neurovascular island flap.

Various vascular models have been used by microsurgeons to practice microsurgical techniques including rabbit ear vessels, the rat renal pedicle, and the rat carotid artery. However, the most useful model has proven to be the lower abdominal skin of the rat where the blood supply is from and to the inferior epigastric artery and vein (the first superficial branches distal to the inguinal ligament). In this model the skin flap is totally supplied by the epigastric blood vessels. Using this skin area it is possible to excise and maintain an island skin flap as large as 8 X 5 cm.

Inbred Lewis rats (which are inexpensive and easy to obtain) were initially used for the development of the surgical technique while the nude rat colony was being established. Syngeneic skin for grafting was taken from one rat, the hair removed, and the skin keratomed to a 0.5 mm thickness and then divided into four or six grafts. Each graft was then subcutaneously implanted under the abdominal skin of the host rat with the epidermal surface of the graft facing the abdominal musculature and the subcutaneous surface facing the subcutaneous layer of the host skin, creating a "sandwich" of host-graft skin. Ten to fourteen days later the sandwich flap was elevated and isolated on the inferior epigastric vessels.

The isolation of the vascular pedicle was accomplished by exposing the femoral and superficial inferior epigastric vessels through a transverse incision in the groin. The femoral artery and vein are dissected distally from a point under the inguinal ligament to about 10 to 15 mm beyond the

Table A  
Summary of Nude Rat Stock  
(October 20, 1983)

Strain	Breeder Pairs	Age			
		< 4 weeks	4-6 weeks	6-8 weeks	> 8 weeks
Outbred (NCI & NIH)	12	1 male 3 new litters (# of nude to be deter- mined)	3 male 2 females	0	12 males 4 females
Lewis (Stanford)	1 male 4 females (one cage)	0	0	0	0
Rowett (Sprague- Dawley)	10	1 male 2 females 3 new litters (# of nude to be determined)	0	2 males	3 females

profunda junction. The superficial interior epigastric vessels are dissected for ten millimeters from their junction with the femoral vessels. In this procedure the nerves accompanying the blood vessels are preserved. All branches of the exposed femoral vessels are ligated and cut with the exception of the superficial interior epigastric vessels. A side by side arterial-venous shunt is constructed between the distal stumps of the femoral artery and vein. This is accomplished by making an elliptical incision in the arterial and venous walls which allows a better pass through of the blood flow and prevents vasoconstriction at the site of the shunt. The human-rat skin neurovascular island flap (flap model) is then transferred to the back to protect it from being eaten by the rat. An incision is made through the skin of the back. The flap is then moved through a subcutaneous tunnel to the opening in the back and sutured in place.

The previously described model has been successfully established on athymic nude rats with an average weight of 300 grams. A summary of the procedure is presented in Figure A.

#### V. Measure blood flow to the flap model.

The measurement of blood flow to the human-rat skin neurovascular island flap is an important factor in evaluating effects of cutaneous injury and determining rates of absorption and metabolism of topically applied agents. Several objectives are being pursued.

##### A. Determine the volume blood flow to the flap model.

Quantitative blood flow through the femoral artery and vein supplying the epigastric vessels of the flap are being measured using an electromagnetic flow meter. The instrument has been calibrated in vitro using excised femoral vessels. In vivo studies using 0.5 mm and 1.0 mm externally applied C-type flow probes have been performed on both the femoral arteries and veins. The flow data is reviewed in Table B. Correlations between electromagnetic blood flow values and skin blood flow measured by laser Doppler velocimetry (LDV) are in progress. Decreases or increases in electromagnetic flow values are simultaneously reflected by increases or decreases in LDV values. We are now beginning to perform the bulk of our experiments on nude rats and simultaneous measures of electromagnetic blood flow through the femoral vessels, LDV measures of skin blood flow velocity, and systemic blood pressure will be recorded during percutaneous absorption studies. This will allow us to account for the effect of these variables on percutaneous absorption and response to cutaneous injury.

##### Objectives for continued experimentation:

1. Establish the relationship between electromagnetic flow values and LDV flow velocity values.
2. Evaluate blood flow velocity in a control site of normal rat skin and in the skin of the flap model.

##### B. Evaluate blood flow velocity at a control site and in the skin of the model flap.

Norms are being established for circulation through the normal abdominal

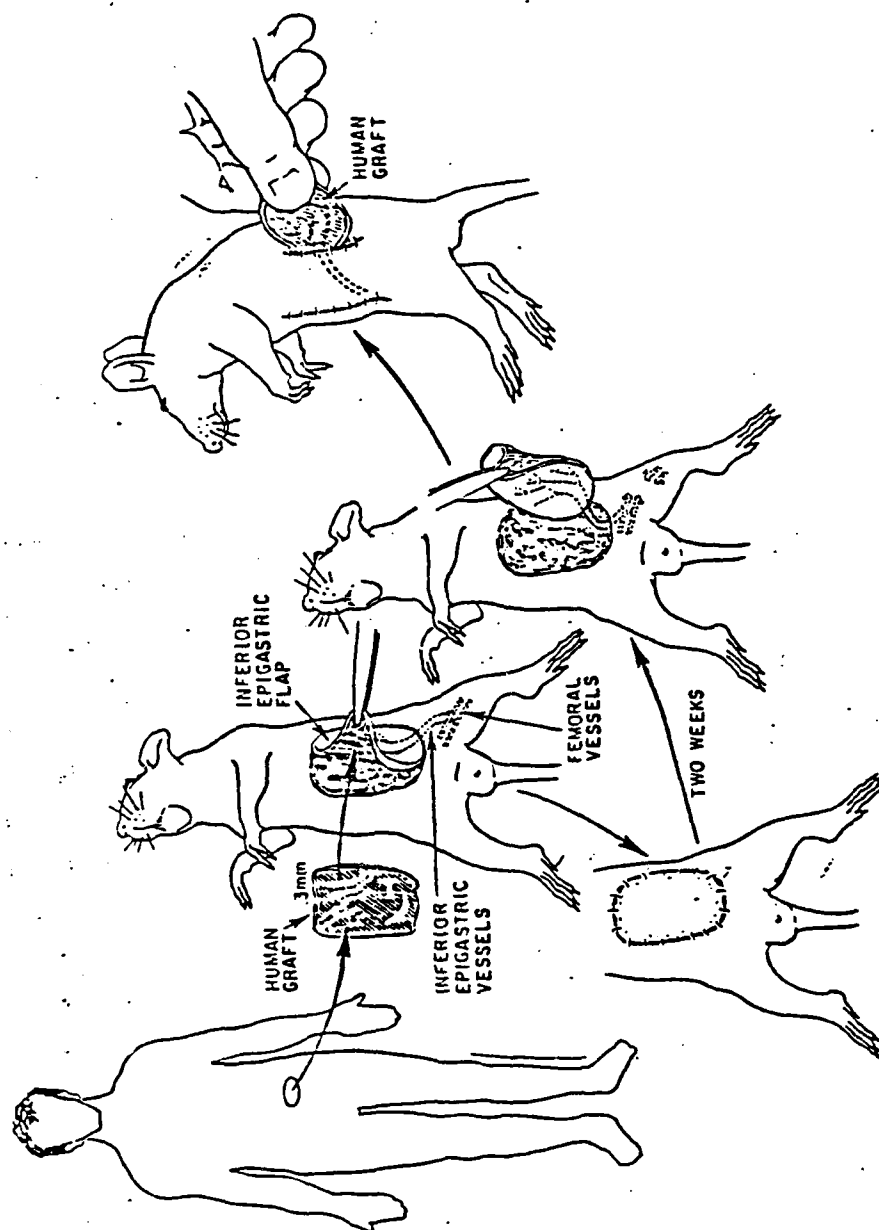


Figure A

Development of Neurovascular Island Flap Model

Table B  
Electromagnetic Blood Flow Values  
Lewis Rats 300-400 gm  
n=9

	<u>Femoral Vein</u>	<u>Femoral Artery</u>
Mean flow	1.86 ml/min	1.91 ml/min
Range	1.0-2.5 ml/min	1.25-2.5 ml/min



skin of the rat as well as to the host and graft side of the flap using the LDV. The complex architectural organization of skin blood vessels has significantly contributed to the difficulty of non-invasively measuring skin blood flow. A relatively new instrument, the laser Doppler velocimeter, overcomes many of the microcirculatory measurement problems, and provides a non-invasive continuous assessment of cutaneous blood flow velocity on any tissue surface. The LDV uses a helium-neon light source to collect frequency shifted, back scattered light, from which arbitrary velocity values expressed in millivolts are derived according to the Doppler principle. The instrument has provided reliable measures of skin blood flow throughout our experimental procedures.

Blood flow to normal abdominal skin serves as a control for monitoring skin blood flow during experimental procedures. The normal abdominal skin is supplied by the inferior epigastric artery. In 300-350 gram rats, anesthetized with nembutal and with normal body temperature, the blood flow is approximately 300-350 ( + 100) m.v. A similar value is obtained from the host side (rat skin) of the sandwich prior to the elevation and transfer of the flap. Immediately after the elevation and isolation of the flap on the vascular pedicle, the blood flow measured by LDV decreases significantly to approximately 70-100 m.v. The exact cause of this decrease in flow is unknown. The LDV values can be increased by bathing the femoral and epigastric blood vessels in a 0.01% solution of xylocaine or in warm saline. Blood flow is significantly restored within twenty-four hours and appears to be stabilized within ten to fourteen days (Table C ). Further studies are being conducted to determine what factors acutely influence flap blood flow. We plan to monitor systemic blood pressure by use of a rat tail sphygmomanometer to determine relationships between systemic blood pressure variations and flap blood flow. Preventing a prolonged decrease in flap blood flow post-operatively promotes flap viability and allows performance of experimental procedures on the flap the same day the flap is created.

Objective for continued experimentation:

Determine the effect of changes in systemic blood pressure on flap blood flow.

C. Document blood flow variations between human and rat skin sides of the model flap.

Blood flow through the human skin side of the flap is approximately 30% - 40% less than the rat skin side of the flap. Two morphologic techniques will be implemented to define the relationship between vessel structure and density, and blood flow variations between the host and graft sides of the flap. The two techniques include barium latex vessel injection radiography and india ink staining for histologic analysis. Such data will provide insights to rates of percutaneous absorption, and responses to different types and degrees of cutaneous injury.

Objectives for continued experimentation include:

1. Describe the morphology of the model flap circulation.
2. Determine the development of angiogenesis or vessel atrophy with exposure to different chemical agents or types of cutaneous injury.

Table C  
LDV Grand Means (m.v.) by Flap Age  
Lewis Rats

<u>Age</u>	<u>Graft Side</u>	<u>Host Side</u>
1 day	166	197
1 week	187	224
2 weeks	132	247
3 weeks	144	234
2-3 months	180	253
5+ months	<u>190</u>	<u>218</u>
Weighted mean	153 (67% of host)	223

D. Determine how blood flow through the model flap varies with changes in body core and flap temperatures.

Changes in core body temperature influence blood flow rates through normal abdominal rat skin as indicated in Figure B. A study of the effects of temperature variation on flap blood flow is currently being conducted. It appears that blood flow changes predictably with changes in temperature. If this relationship is valid we may be able to regulate blood flow to the flap without pharmacologic or mechanical interventions by simply controlling the animal's body temperature. Further blood flow investigations will also include cooling the flap while maintaining normal body temperatures, and cooling the animal while maintaining normal flap temperatures.

Objective for continued experimentation:

Establish the relationships between variations in temperature, skin blood flow, and rates of percutaneous absorption for different agents.

E. Determine the extent of collateral circulation established between the model flap and the surrounding tissues.

The determination of the amount of blood flow supplied by collateral circulation to the flap is important to the understanding of percutaneous absorption in our systems. The interface between the flap and the back skin of the rat has been made as small as possible for structural control. A protocol has been developed for evaluating collateral circulation using a prototype dermofluorometer and the LDV. The distribution and clearance of intravenously administered sodium fluorescein is an established method of documenting nutrient blood flow to the skin. The dye fluoresces yellow-green on illumination with blue light. Previous methods of assessing tissue fluorescence have been limited to visual inspection or photography. The dermofluorometer has been developed to non-invasively quantify tissue fluorescence using considerably smaller amounts of fluorescein than required for qualitative evaluation of fluorescence.

The dermofluorometer transmits wavelengths of light between 450 and 500 nanometers. This wavelength stimulates maximal excitation of sodium fluorescein in vivo. Fluorescent emission from the tissue is transmitted through a fiberoptic interface between the end of the instrument probe and a photomultiplier tube. A digital readout in volts provides a quantitative measure of fluorescence. Higher values represent greater saturation of tissue with fluorescein. Comparison of clearance curves in experimental and control sites provides an index of skin blood flow.

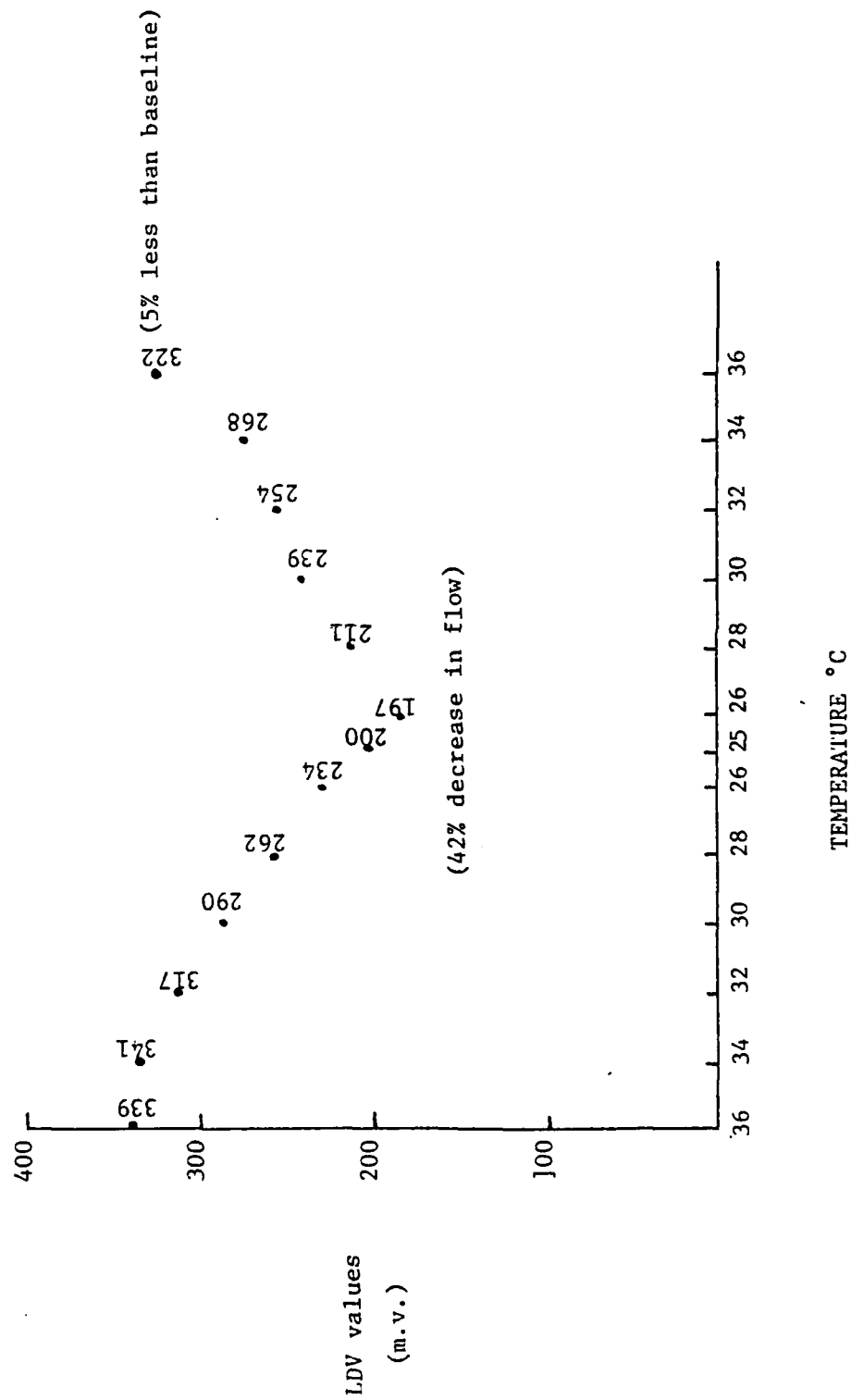
The dermofluorometer was used to determine collateral circulation to neurovascular islands flaps in Lewis rats. The evaluation was approached by clamping the inferior epigastric artery which is the major arterial supply to the flap. Any remaining circulation would be from a collateral vascularization. Fluorescein was then injected intravenously followed by repeated dermofluorometry measures on the flap and at an abdominal control site. LDV measures were simultaneously obtained. The results indicate the collateral circulation is adequate to sustain viability of the flap when the epigastric artery is ligated. Predictive parameters for determining the extent of the collateral circulation are being established for the nude rat.

LDV Change With Change in Core Temperature

(Lewis Rats - Normal R Abdominal Skin)

n=5

Figure B



We anticipate it may be necessary, for certain studies, to surgically isolate the blood supply to the flap with each percutaneous absorption study.

F. Develop a method for accessing blood flow to and from the model flap.

Several procedures for establishing a vascular access have been analyzed for sampling the blood flowing to and from the flap. Different bore sizes of silastic tubing ranging from 0.2 mm to 0.8 mm in diameter have been placed in the femoral arterial-venous anastomosis. Clotting and displacement of the tube have been persistent complications. Systemic heparinization with an implanted osmotic heparin pump was not successful. The most successful approach has been to create a venotomy on the venous side of the anastomosis and sample with a heparinized capillary tube. We are currently evaluating the effect of repeated samplings, on different days, on the integrity of the vessel wall to determine how it may affect blood flow dynamics. Administration of substances is easily accomplished using a very sharp 30 gauge needle inserted through the anastomosis into the femoral artery.

Objective for continued experimentation:

Continue to evaluate the effects of repeated sampling on vessel structure and blood flow.

VI. Acquire Human Skin for Grafting.

Earlier in the project availability of human skin was a problem. Currently we have a stable supply of readily accessible human skin. Because human skin acquisition may be a potential problem, we are constantly searching for ways of maintaining human skin, or creating equivalents of human skin in a laboratory setting. Collaborative relationships have been established with investigators from the Massachusetts Institute of Technology for evaluating collagen glycosaminoglycans sheets as a substrate for epidermal sheets or epidermal cells grown from tissue culture. The thrust of our efforts are being directed toward technology for developing epithelial cells using a serum free system. The results of this investigation will contribute to evaluation of cutaneous injury and percutaneous absorption in the human-rat skin model.

VII. Graft Human Skin to the Nude Rat.

The first trials of grafting human skin to nude rats resulted in rejection of the human skin. The mechanism by which this is accomplished is unknown. A series of experiments to more carefully define this process has been undertaken. To date the experiments include:

A. Determining that rejection is not secondary to grafting technology.

This problem was approached by implanting allogeneic and autologous rat skin along with human skin of similar thickness to the nude rat. The nude rat will retain his own skin and that of other rats but will reject human skin. This suggests the rejection is not secondary to grafting technology. Various thicknesses of human skin have been grafted and all have been rejected. Additionally we have analyzed various procedures for the placement of orthotopic grafts including: grafting and covering with Op-Site; grafting subcutaneously and then exposing the skin; grafting subcutaneously and then

isolating the graft on a vascular pedicle and suturing the graft in place. None of these variations have prolonged graft survival.

At this point it appears as though the graft rejection starts at about three weeks after grafting and is complete within ten days. Thereafter, if the animal is rechallenged with a human skin graft, it is rejected within seven to ten days. Some grafts have remained in place for seven to eight weeks before undergoing rejection. This may be related to the ages of the animals. If animals are grafted at approximately three weeks of age, it appears as though the graft survives for longer periods of time.

The histology of the graft shows a typical effacement of the basal membrane, with subsequent degeneration of keratinocytes and influx of inflammatory cells. There is a immunoglobulin deposit that is heavy within the stratum corneum and is largely IgM. We are in the process of obtaining better reagents to more clearly define the antibody depositions. Studies to define the infiltrating cell type have not been undertaken.

Despite these negative findings, we have been encouraged by the results of three grafts. Two, which are parts of grafts, have been in place for more than 16 weeks. A third has been in place in total for more than twelve weeks. These positive results lead us to believe the rejection problem can be overcome.

#### B. Defining the immune status of the nude rats.

Autopsies have revealed the nude rats do not have a thymus gland, with no evidence of a thymic component within their spleen or lymph nodes. Further, grafting the animal does not seem to restore thymic competence relative to response to concanavallin A. Likewise, there are no thymic epithelial centers on post-mortem examination of the lymph nodes and spleen of grafted animals. Interestingly, only the grafted animals display specific cytotoxicity to human cells. This phenomena is rather impressive and is only seen when lymphocytes from lymph nodes are incubated with human peripheral blood mononuclear cells for four days and then assayed for their ability to lyse chromium labeled human peripheral blood mononuclear cells.

Neonatal tolerance has been attempted by implanting human skin, subcutaneously, in litters of newborn rats. These experiments were controlled by placing the same types of grafts in animals that are immunologically competent, i.e. the normal litter mates. The skin grafts were implanted at three days of age. These animals recently have been regrafted, and all animals have rejected the human skin grafts somewhere between the sixth and ninth week of ingraftment.

#### Objectives for continued experimentation:

- a. Further definition of the role of the cytotoxic lymphocytes that develop in the grafted nude rat, i.e. do they play a role in graft rejection.
- b. Determining what T-independent antigens are recognized by either antibody mediated systems or "natural" killer lymphocytes.
- c. Determining whether or not treatment with anti-IgM or radiation at the time of grafting will prevent rejection.
- d. Grafting with skin that has a higher vascularity, i.e. face-lift skin,

rather than abdominal skin.

e. Grafting with different thicknesses of skin.

#### VIII. Assessing Epidermal Cell Proliferation Following Injury:

A basic hypothesis, in need of testing, is that alterations in epidermal cell proliferation occurs following cutaneous injury. The proliferations is manifest clinically as scale and hyperproliferation. In vitro there is an increased epidermal cell turnover, i.e. an increased labeling index using autoradiography. Contrawise, there can be a decreased proliferation following injury, i.e. epidermal thinning, and a decreased labeling index. Current systems use alterations in 3-H thymidine incorporation into the epidermis. This method is time consuming and difficult. Therefore, we are working on a system that is less cumbersome and yet provides a precise quantitative assessment of epidermal DNA synthesis. Our goal is to be able to quantitate DNA synthesis of any cell in a frozen vertical section of skin that has undergone experimental manipulation. Currently, we are developing a technique which uses bromodeoxyuridine (BrdUrd) as the precursor nucleoside and its well-known ability to quench the fluorescence of the vital dye, H-33258, when activated with an appropriate wave length of light.

The experimental design is to incubate the test skin with BrdUrd, snap freeze, prepare a cryostat section, and counterstain the section with H-33528.

Cells recently synthesizing DNA, BrdUrd containing, do not fluoresce as brightly as cells that have not synthesized DNA in the presence of BrdUrd when the tissue is activated by a fluorescent light source. The difference in fluorescence can be quantitated with a micro-fluorometer attached to the microscope. The degree of quenching is proportional to the amount of new DNA synthesized. A computer program has been developed which allows a quantitative measure of this quenching effect in a very short period of time for each cell, less than four seconds. Figure C is a demonstration of a histogram generated by incubating a squamous cell carcinoma cell line in BrdUrd for two periods of time and then analyzing the cells with the foregoing methodology. As can be appreciated, the longer incubation time results in more cells having undergone DNA synthesis and the quenching changes accordingly. Figure D is a correlation between 3-H thymidine incorporation (grain counts) and the degree of quenching. This methodology is now being adapted to skin. We have early evidence that epidermal cells and vertical frozen sections of foreskin incubated with BrdUrd can be analyzed for DNA synthesis using this technology. The significance of the technology is related to the speed of assessing experimental results. Data for analysis is available within 24 hours of completing the experiment as a print out of a histogram quantitating the quenching (DNA synthesis) of BrdUrd. If this technology works as expected, it is very likely it will be adapted to the experimental protocol in our study of cutaneous injury.

#### Discussion

The surgical technique for creating the model flap has been successfully developed using syngeneic skin in Lewis and nude rats. The grafts have remained healthy and viable throughout the life of the animals, providing an experimental skin surface for long term evaluation of topically applied agents or cutaneous injury. Although initial experiments of grafting human skin by

Figure C

INCREASE IN H-33258 DYE FLUORESCENCE  
 & BROMODEOXYURIDINE INCORPORATION (BrdUrd)  
 (↑ VOLTAGE CORRELATES & BrdUrd UPTAKE)

CELL CULTURE: HUMAN SQUAMOUS CELL  
 CARCINOMA LINE (SCL-125)  
 BrdUrd DOSE: 20  $\mu$ M  
 [DYE] = 1.0  $\mu$ g/ml  
 \* VALUES REPRESENT RANDOM  
 EVALUATION OF 40 CELLS/EXPERIMENT

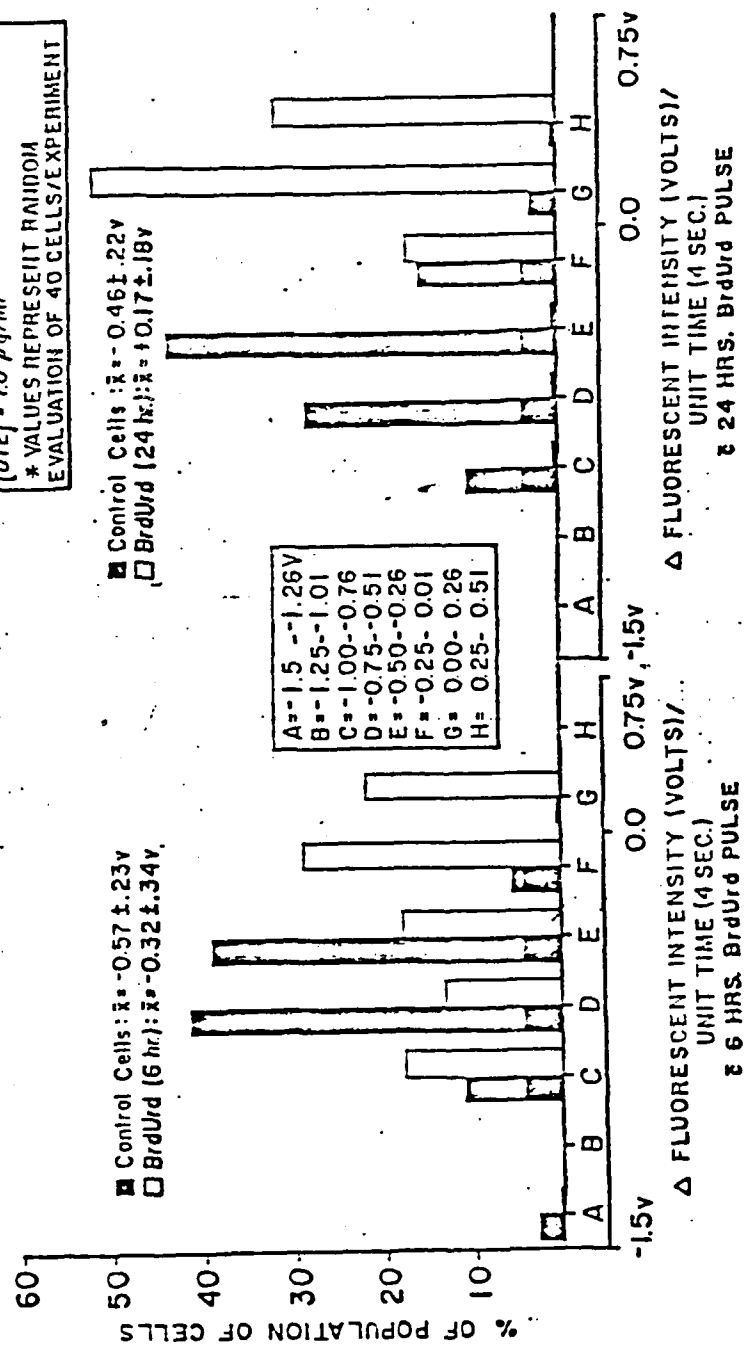
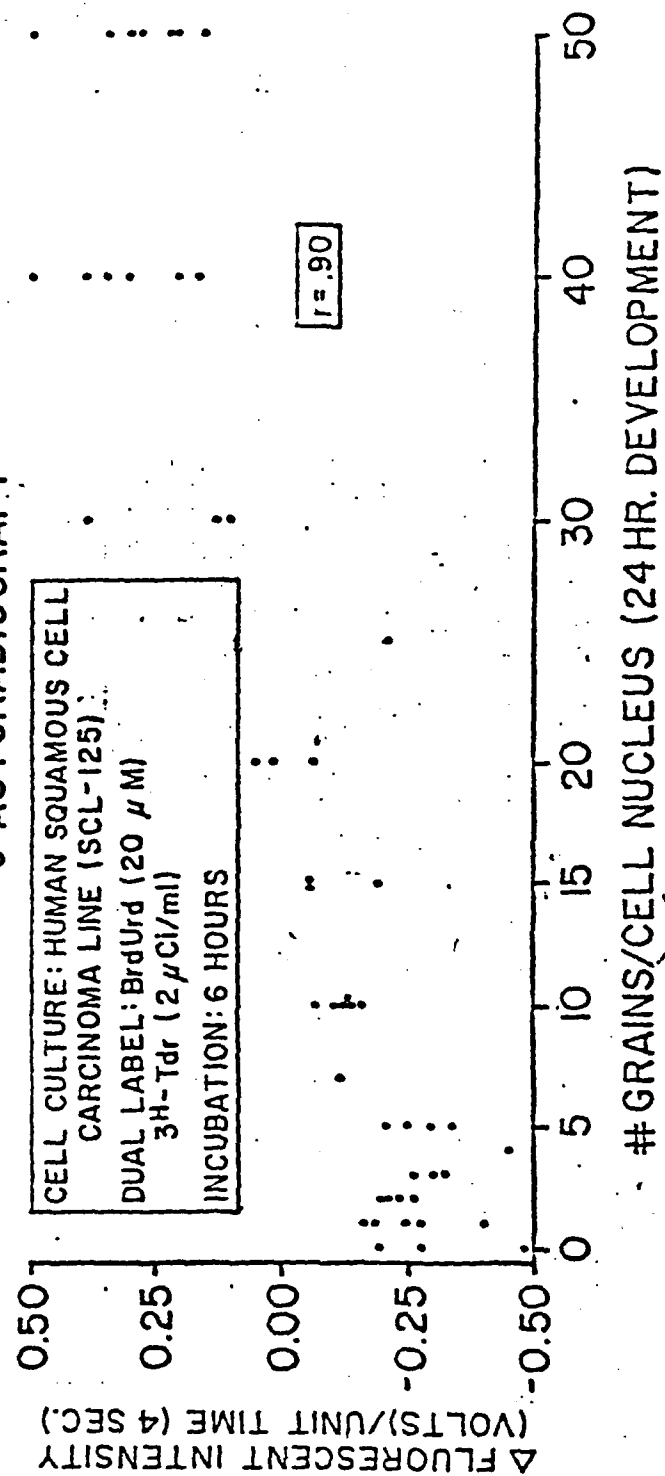




Figure D  
 CORRELATION OF BrdUrd FLUORESCENCE  
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the nude rats resulted in rejection, the retention of human skin grafts in three animals provides hope that the grafting procedure will be successful. We are optimistic that the rejection process can be defined and controlled.

Volume blood flow to the flap can be measured using externally applied electromagnetic flow probes, in spite of the small vessel size. Small diameter probes have been designed at 0.5 mm and 1.0 mm for flow measurements in the femoral artery and vein. In vitro calibration studies using flow through probes and C-probes in excised vessels has helped to establish the validity of measurements. The laser Doppler velocimeter with its non-invasive probe design and continuous measure of skin blood flow has proven to be a sensitive and reliable indicator of changing skin blood flow.

Techniques for evaluating rates of percutaneous absorption have been defined and will be initiated using radiolabeled benzoic acid. Variables such as graft thickness, blood flow, body temperature, and vasoactive agents will be evaluated.

A perfection of methodology for evaluating cutaneous injury is currently being defined in normal rats and will be implemented for the nude rat model flap system once the human skin rejection problem has been resolved.

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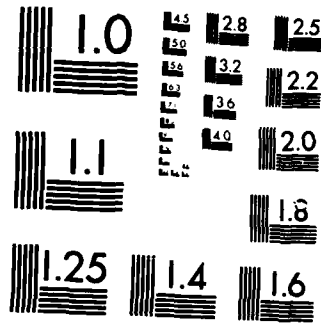
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**INFORMATION**

*Correction*

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
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18. SUPPLEMENTARY NOTES None		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Human skin graft on athymic rat Human skin model to study percutaneous penetration and cutaneous injury		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A model for grafting human skin to a neurovascular island flap is being developed on congenitally athymic nude rats. Success with the model will provide a human skin surface for the study of chemical warfare agents. Applying microsurgery techniques, a neurovascular island flap using syngeneic skin has been successfully established in both the Lewis and nude rats. Early experimentation using human skin for the flap model in nude rats resulted in rejection. The immunologic mechanisms responsible for the rejection phenomenon are unknown. A series of experiments has been initiated to define factors		



## Continuation of block 20:

causing the rejection process. Grafts have remained viable in three animals for 12-16 weeks indicating the rejection problem may be resolved.

An evaluation of blood flow dynamics has been undertaken since blood flow is significantly related to rates of percutaneous absorption and skin responses to cutaneous injury. Volume blood flow measurement through the vessels supplying the flap is being established by use of C-type electromagnetic flow probes, which are applied to the outside of the vessel. Preliminary measures in Lewis rats indicate the flow rate is between 1.0 and 2.5 ml per minute. The evaluation of skin blood flow at control sites and on both sides of the flap is being assessed using laser Doppler velocimetry. LDV values at abdominal control sites are approximately 300-350 m.v. Blood flow to the host side of the graft is less than the control site and approximately 60% greater than the human skin graft. Blood flow to the flap is more than required for maintaining flap viability. Investigations manipulating blood flow through the flap are being initiated and include an evaluation of blood flow change with changes in body and flap temperature, and use of vasoactive agents.

Histologic techniques for assessment of cellular responses to cutaneous injury are in process. The techniques are related to quantitatively assessing epidermal DNA synthesis.

Continuing experimental objectives have been projected to include:

- a. Defining relationships between electromagnetic flow values, LDV flow velocity values, and systemic blood pressure.
- b. Describing the morphology of the model flap circulation.
- c. Determining the effects of repeated blood sampling on vessel structure and blood flow.
- d. Defining the rejection process of the human skin grafts by the nude rat.
- e. Evaluate relationships between percutaneous absorption, cutaneous injury, and different thickness and types (facial vs abdominal) of skin.
- f. Quantitate responses to cutaneous injury.

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